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Original Article

Anodic Stripping Voltammetry Determination of Total Arsenic in Urine Using Gold Rotating Disc Electrode: A Method Validation

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Abstract

The salts of Arsenic (As) are of great toxicological importance and can cause poisoning. The quantitative determination of traces of arsenic and its compounds is important to assess its harmful levels in environmental and biological samples. Therefore quantitative determination of traces of Arsenic in urine is very essential. Routinely, inductively coupled plasma, atomic absorption spectrometry, graphite furnace atomic absorption spectrometry were used for analysis of Arsenic. An attempt has been made to develop for determination of traces of Arsenic in urine using anodic stripping voltammetry. The analysis utilizes three electrode systems, Gold rotating disc electrode (RDE) as a working electrode, Ag / AgCl as a reference electrode and Glassy carbon electrode as an auxillary electrode. Urine was processed by closed digestion method using 34.5% nitric acid (HNO_3). Determination of Arsenic was made by primary solution with a sweep rate of 20mV / s and pulse amplitude 50 mV by standard addition method. The solution was purged with nitrogen gas and cleaning was done at -1200 mV for 60 seconds and the potential was scanned from 300 mv to 400 mv on RDE with stirrer speed 2000 rpm. As (III) and As (IV) in HCl were reduced at -1200 mV by nascent hydrogen to As^0 and was deposited at 0 mv for 10 sec. The deposited metal was sweeped by scanning the potential from -200 mV to 300 mV using DP mode. The stripping current arising was correlated with the concentration of the metal in the sample. The peak potential for Arsenic is 50 mV. The detection limit of Arsenic by this method was 1.0µg / l.

Keywords: Anodic Stripping Voltammetry (ASV); Gold Rotating Disc Electrode; RDE; Arsenic; Urine; Glassy Carbon Electrode.

Introduction

Arsenic is widely distributed throughout Earth's crust, generally as arsenic sulfide or as metal arsenates and arsenides. The normal level of arsenic in whole blood concentration should be less than 50µg / L. Level of arsenic in urine measured in a 24 hour collection, following 48 hours without eating seafood exceeds 100µg / L in people with arsenic poisoning. In nails and hair it should be less than one ppm [1-3]. Drinking Arsenic contaminated water poses a variety of health problems such as skin lesions include melanosis, leucomelanosis and keratosis. Other problems are high blood pressure, diabetes mellitus, lung disorders and peripheral neuropathy [4-8].

Arsenic has been very popular from centuries as a homicidal agent. It can cause instant poisoning and death if consumed in large amounts. In cases of acute poisoning it causes nausea, vomiting and diarrhea1. Chronic exposure to Arsenic may lead to hyperkeratosis and skin pigmentation. Dermal

exposure causes arsenicosis which is the first symptom of arsenic exposure. Dermatitis is common in arsenic exposure. When it is inhaled it causes primarily the lung cancers and secondarily the liver, skin and digestive tract cancers have also been observed through various studies. Areas where water is contaminated of Arsenic, skin tumors are the most common types of cancer. Oral exposure increases the risk of other kinds of cancer such as bladder, lung, liver, kidney and prostate. Arsenic is also known to cause cancer of skin, lung and bladder [9-12].

Arsenic exists in the environment in two forms arsenic (III) and arsenic (IV). Among these two, arsenic (III) is the more potent and hazardous [13-14]. Due to its toxic effects arsenic is also known as the "king of poisons". Through water it is transported mainly by blood [15], after entering into the blood, As (III) undergoes methylation inside the hepatic cells. It is methylated to form MMA (V) which further undergoes reduction to form MMA (III). It undergoes subsequent oxidative methylations to form DMA (V) which is the primary excretory product after arsenic exposure [16].

Measuring total amount of arsenic in urine is the most reliable means of detecting recent arsenic exposures [17]. The tests conducted in urine gives total amount of arsenic present in the body. Routinely, inductive coupled plasma, atomic absorption spectrometry, graphite furnace atomic absorption spectrometry have been used for the analysis of arsenic [18-21]. An attempt has been made to develop a new method for determination of total arsenic in urine by using Gold Rotating Disc Electrode (RDE). One of the major advantages of this technique is that the running cost of instrument is low, compared to any other technique. In the present study, determination of arsenic was made in hydrochloric acid medium with a sweep rate of 20 mV/s and pulse amplitude 50 mV by Gold Rotating Disc Electrode.

Materials and Methods

Apparatus and Accessories

1. Trace metal analyzer model 797 VA Computrace from Metrohm AG Ltd, Switzerland (Fig. 1) was used, which contains following electrodes:

Working Electrode	- Gold Rotating Disc Electrode (RDE) (Fig .2)
Auxillary Electrode	- Glassy Carbon (Fig. 3)
Reference Electrode	- Ag/ AgCl (Ammonia buffer)

2. Nitrogen gas of purity 99.99% from laser gases, India was used.
3. Micropipette of Eppendorf make of volume 10 - 100ml and 100- 1000 ml were used.

Reagents and Chemicals

Fig. 1: 797 VA Computrace (Trace Metal Analyzer from Metrohm)

Fig. 2: Rotating disc Electrode (RDE) (From Metrohm)

Fig. 3: Glassy Carbon Electrode (From Metrohm)

Suprapur hydrochloric acid (HCl), 1000 ppm arsenic standard solution (traceable to SRM from NIST H2AsO3 in HNO3, 0.5mol/l) from Merck, Germany and Ultrapure water from Rion India were used.

Glasswares

Volumetric flask of 50 ml capacity from Borosil India was used. The glasswares were thoroughly washed and rinsed 2-3 times with ultrapure water and dried in digital oven.

Urine Sample

Urine sample of the OPD patient of AIIMS New Delhi was used.

Preparation of 1 ppm Standard Arsenic Solution

1 ppm solution of arsenic was prepared by diluting 1000 ppm of standard solution.

Preparation of 30% HCl solution

30 ml of suprapure HCl was taken in 100 ml volumetric flask and make up to mark.

Preparation of 34.5% HNO_3

Equal volume of concentrated nitric acid and ultrapure water were mixed.

Sample Preparation

Liner vessels of microwave digestion system were cleaned with HNO_3 and water mixture (1:1) followed by water and dried. 10 ml of urine sample was placed in the liner vessel. 15 ml of 34.5% HNO_3 was added in each liner vessel in fume hood. In the reference vessel, 1 ml of water (instead of sample) was added along with 15 ml HNO_3 for sample blank. Vessel carrousels were loaded in the microwave digestion oven and the microwave was run with the program given in Table 1. After digestion, the samples were allowed to cool down and then, each vessel was opened in the fume hood. Digested sample was transferred to 50 ml volumetric flask and then the volume was made up to the mark with the help of ultrapure water.

Conditioning of the Gold Electrode

Table 1: Programming Condition for Microwave Digester

Step	Time (sec)	Starting temp (^{0}C)	Ending temp (^{0}C)
1	200	28	100
2	400	100	160
3	400	160	170

In order to obtain reproducible curves the gold electrode must be electrochemically conditioned. This should be done every day before starting the measurements and also when the standing current varies strongly from measurement to measurement. Generally the standing current should as small as possible, which should be between 0.5 and 1.5 µA at -200mV. For conditioning 10 ml of ultrapure water and 1ml of 30% of HCl was taken in voltammetric vessel of the instrument and conditioning was done under the condition given in the Table 2.

Determination of Blank Value of the Reagents

Table 2: Parameter for conditioning of gold electrode

Parameter	Value
General	
Working electrode	RDE
Stirrer speed	2000 rpm
Purge time	300 s
Conditioning Cycle	
Cleaning potential 1	-1500 mV
Cleaning time 1	30 s
Cleaning potential 2	+ 400 mV
Cleaning time 2	60 s
No. of repetition cycles	16
Sweep	
Equilibration time	5 s
Start potential	-200mV
End potential	+ 300 mV
Voltage step time	0.3 s
Sweep rate	20 mV/s

It is very difficult to obtain reagents that are completely arsenic free. The content can also vary from batch to batch. Owing to the high sensitivity of this method it is therefore absolutely necessary to determine the blank value of the reagents used. This was determined in 10ml ultrapure water +1 ml 30% of HCl solution in the same way as the arsenic content of the sample is determined.

Anodic Stripping Voltammetry (ASV) Determination of Arsenic

The electrode was washed with ultrapure water. 10 ml of ultrapure water and 1 ml 30% HCl were taken in voltammetric vessel and voltammogram was recorded under the condition given in Table 3. After completion of voltammogram, 0.1 ml of digested urine sample was added in voltammetric vessel and voltammogram was recorded under same condition. After completion of the sample Voltammogram, 0.1 ml of 1 ppm standard solution of As was added and voltammogram was recorded. Again, 0.1 ml of 1 ppm standard solution was added in the same vessel and voltammogram was recorded second time. The concentration of the analyte was calculated by linear regression method (standard addition). All the measurements were done by standard addition technique to avoid the sample matrices effect. The voltammogram of the standard and sample was given in fig. 4(a) and 5(a) along with their extrapolation graphs 4(b) and 5(b).

Table 3: Parameters for ASV Determination of Arsenic

Parameter	Value
General	
Working electrode	RDE
Stirrer speed	2000 rpm
Mode	DP
Purge time	130 s
Deposition	
Cleaning potential (Deposition potential 1)	-1200 mV
Cleaning time (Deposition time 1)	60 s
Deposition potential (Deposition potential 2)	0 mV
Deposition time (Deposition time 2)	10 s
Sweep	
Pulse amplitude	50 mV
Start potential	-200mV
End potential	+300 mV
Voltage step	6 mV
Voltage step time	0.3s
Sweep rate	20 mV/s
Pulse amplitude	50mV
Pulse time	0.40s
Peak potential As	+50 mV
Equilibration time	5 s

Result and Discussion

Voltammetry is a technique in which the current flowing through an electrode is measured while a potential scan is superimposed on that electrode. Voltammetry is a three electrode system consisting of a working electrode, a reference electrode and an auxillary electrode which involves two steps. In the first step metals / metal ions are deposited on the electrode and in the second step metals / metal ions are stripped out of the electrode. The metal / metal ions stripped out are directly proportional to the current, greater the current greater will be concentration of metals and lesser will be current lesser the concentration of metals. The total arsenic content is determined at a gold electrode with the electrode surface on its sides. As (III) and As (IV) in HCl were reduced at 1200 mV by nascent hydrogen to As^0 and deposited. Deposited metals are anodically striped by scanning the potential from -

200 mV to 300mV. All the measurements done by standard addition technique in which first sample is taken into the voltammetric vessels and current is measured, then standard of known concentration is added twice to the sample solution and the current is measured. After all the measurements extrapolation curve is plotted between current vs. concentration. The extrapolation curve will show the amount of metals present in the sample solution. All the analysis was done with automatic blank subtraction, which is feature of the instruments.

Voltamogramme of As obtained from standard addition technique is given in (Fig. 4(a) and 5(a). The sensitivity was calibrated by standard additions to the sample and the initial metal concentrations were calculated by extrapolation given in Fig. 4(b) and 5(b). Consequently, the linear calibration range was automatically obtained as being related to quantitative mode of the Voltammetric unit. Under these conditions, Concentration of arsenic in the sample was 116.75µg/L. Several papers [10, 11, 18] have discussed the determination of Arsenic in matrices other than the urine. The advantages of proposed voltammetric method over the other known techniques include the sensitivity of the method besides the other features such as the rapidity, cost effectiveness and sophistication of the method.

Fig. 4a: Voltammogram of Arsenic Standard

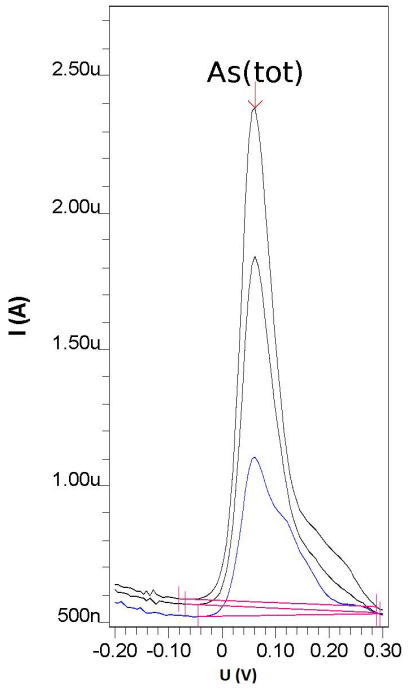


Fig. 5a: Voltammogram of urine sample

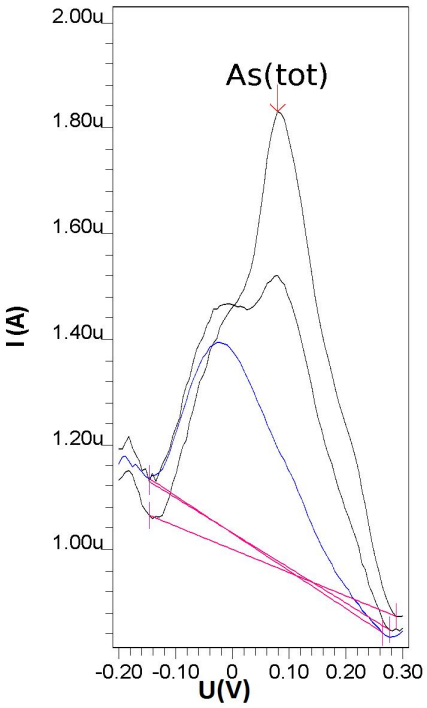


Fig. 4b: Extrapolation graph of Arsenic standard

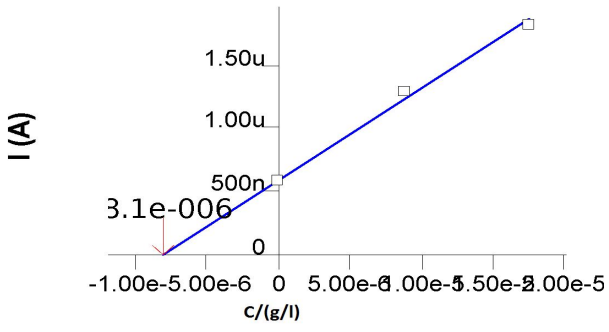
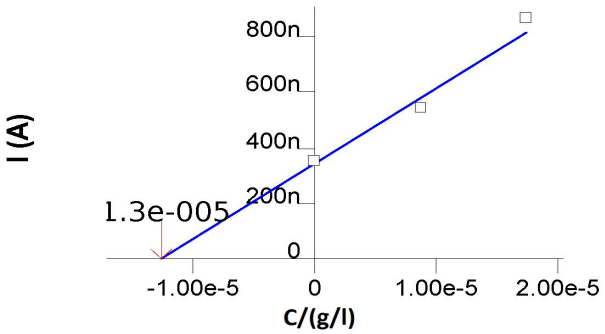


Fig. 5b: Extrapolation graph of urine sample



Conclusion

This article describes the determination of total arsenic by anodic stripping voltammetry (ASV) using gold rotating disc electrode (RDE). A determination limit of 1.0 µg/l was achieved with 10 ml sample solution. The total arsenic content is determined at a gold electrode with the electrode surfaces on its sides. As (III) and As (IV) in HCl were reduced at -1200 mV by nascent hydrogen to As^0 and deposited. If the deposition is carried out at -200 mV then only As (III) is reduced which allows the differentiation between total arsenic and As (III). The Trace metal analyzer is advantageous in terms of the range of concentration to which it can measure. It becomes a useful technique as it can be used for the analysis of biological samples such as urine.

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